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**EVALUATION OF 3D-HUMAN SKIN EQUIVALENTS FOR ASSESSMENT OF
HUMAN DERMAL ABSORPTION OF SOME BROMINATED FLAME
RETARDANTS**

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Abstract

Ethical and technical difficulties inherent to studies in human tissues are impeding assessment of the dermal bioavailability of brominated flame retardants (BFRs). This is further complicated by increasing restrictions on the use of animals in toxicity testing, and the uncertainties associated with extrapolating data from animal studies to humans due to inter-species variations. To overcome these difficulties, we evaluate 3D-human skin equivalents (3D-HSE) as a novel *in vitro* alternative to human and animal testing for assessment of dermal absorption of BFRs. The percutaneous penetration of hexabromocyclododecanes (HBCD) and tetrabromobisphenol-A (TBBP-A) through two commercially available 3D-HSE models was studied and compared to data obtained for human *ex vivo* skin according to a standard protocol. No statistically significant differences were observed between the results obtained using 3D-HSE and human *ex vivo* skin at two exposure levels. The absorbed dose was low (less than 7%) and was significantly correlated with log K_{ow} of the tested BFR. Permeability coefficient values showed increasing dermal resistance to the penetration of γ -HBCD > β -HBCD > α -HBCD > TBBPA. The estimated long lag times (> 30 minutes) suggests that frequent hand washing may reduce human exposure to HBCDs and TBBPA via dermal contact.

Keywords: Dermal absorption, Human skin equivalents, Human *ex vivo* skin, HBCDs, TBBPA, EPISKIN.

Introduction

Brominated flame retardants (BFRs) are a diverse group of chemicals widely used to prevent or reduce the flammability and combustibility of polymers and textiles. Among the major members of this group are Tetrabromobisphenol A (TBBP-A) and hexabromocyclododecane (HBCD) with estimated global production volumes of 170,000 and 16,700 tons, respectively (BSEF 2014). Since HBCD and ~20% of the produced TBBP-A are blended physically within, rather than bound chemically to polymeric materials; they migrate from products, following which their persistence and bioaccumulative character leads to contamination of the environment including humans (Harrad, et al. 2010). This is of concern owing to their potential toxicological risks including: endocrine disruption, neurodevelopmental and behavioral disorders, hepatotoxicity and possibly cancer (Darnerud 2008; Wikoff and Birnbaum 2011). Such evidence has contributed to several regulations (e.g. REACH) under different jurisdictions to control the production and use of these hazardous chemicals. Recently, HBCD was listed under Annex A of the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP 2014).

Substantial data exist on concentrations of different FRs in various environmental and human matrices (Covaci, et al. 2009; Law, et al. 2014; van der Veen and de Boer 2012). Current understanding is that non-occupational human exposure to BFRs occurs mainly via a combination of diet, ingestion of indoor dust, dermal contact with dust/consumer products, and inhalation of indoor air (Abdallah, et al. 2008a; Frederiksen, et al. 2009; Watkins, et al. 2011). The exact contribution of these pathways varies substantially between chemicals, between individuals according to lifestyle, and is further complicated by international variations in FR use (Abdallah and Harrad 2009; Abdallah, et al. 2008a; Abdallah, et al. 2008b).

Currently, very little is known about dermal uptake as a route of human exposure to BFRs in

indoor dust or flame-retarded products. Watkins et al. reported a significant positive correlation between PBDE levels on hand wipes (presumably resulting from hand contact with contaminated dust or flame-retarded products) and PBDE levels in blood serum from American adults. While concentrations of PBDEs in indoor dust were strongly correlated with those in hand wipes, correlation could not be established directly between PBDE concentrations in indoor dust and their levels in serum (Watkins, et al. 2011). This opens up the possibility that FRs in dust may also be an indicator of another exposure pathway, such as direct dermal uptake of FRs present in treated goods (e.g. games consoles, remote controls, and fabrics). However, the absence of experimental data on human dermal absorption of various BFRs was recently highlighted as a major research gap hampering their accurate exposure assessment. Efforts to fill this gap are currently impeded by several difficulties including: ethical and technical issues inherent to studies involving human tissues, increasing restrictions on the use of laboratory animals in toxicological studies and the substantial uncertainties associated with extrapolating data from animal studies to humans due to inter-species variation (e.g. skin barrier function, hair follicles, intercellular subcutaneous lipids ...etc) (Abdallah, et al. 2015a).

To overcome these difficulties, this study will evaluate the application of *in vitro* 3D-human skin equivalents (3D-HSE) as an alternative method to animal and human testing for assessment of dermal uptake of HBCDs and TBBPA. 3D-HSE are commercially available, fully differentiated, multi-layered dermal tissues that closely mimic the original human skin histologically and physiologically (Schaefer-Korting, et al. 2008a). 3D-HSE consist mainly of primary human cells (e.g. keratinocytes and fibroblasts) obtained from healthy consenting donors, which are then cultured at the air-liquid interphase on a specially designed inert support that allows cell growth in a nutrient culture medium (Figure SI-1). While cells grown in 2D monolayers (e.g. Caco-2 cell models) cannot capture the relevant complexity of the *in*

vivo microenvironment as they lack a myriad of important signals, key regulators, and tissue phenotypes; cells growing in 3D tissue cultures have different cell surface receptor expression, proliferative capacity, extracellular matrix synthesis, cell density, and metabolic functions that resemble closely the original human tissue (Brohem, et al. 2011). Consequently, validated protocols using 3D-HSE models have been approved by the OECD (Organisation for Economic Co-operation and Development) and ECVAM (European Centre for Validation of Alternative Methods) for testing skin irritation, phototoxicity and corrosion by xenobiotic chemicals (Ackermann, et al. 2010; Buist, et al. 2010).

While 3D-HSE have been successfully applied within the cosmetics and pharmaceutical sectors to study dermal uptake of various drugs (Ackermann, et al. 2010; Schaefer-Korting, et al. 2008a), this study of dermal uptake of BFRs, is the first application of 3D-HSE to better understanding of human dermal uptake of environmental contaminants. Our overall objective was to demonstrate the substantial potential of these models to transform how human dermal exposure to such contaminants is assessed. Nested within this, our specific aims were to: (a) develop and apply a standard protocol for assessment of percutaneous penetration of HBCDs and TBBPA using 2 commercially available 3D-HSE models (EPISKIN™ and EpiDerm™) according to the OECD guidelines; (b) compare the results of 3D-HSE models to those obtained from *in vitro* excised human skin (*ex vivo* skin); and (c) provide the first insights into the dermal bioavailability of our target BFRs in humans.

Materials and Methods

Experiments were performed along the principles of good laboratory practice and in compliance with the OECD guidelines for *in vitro* dermal absorption testing (OECD 2004). The handling instructions and performance characteristics of the tested 3D-HSE models were also taken into consideration. The study protocol received the required ethical approval (#

ERN_12-1502) from the University of Birmingham's Medical, Engineering and Mathematics
Ethical Review Committee.

Test matrices.

The EpiDerm™ EPI-212-X human skin equivalent kit was purchased from MatTek Corporation (Ashland, MA). The EPI-212-X tissue constructs are 0.64 cm² human skin equivalents resembling the normal human epidermis histologically and physiologically (www.mattek.com). The kit includes maintenance medium (MM) - which is a proprietary DMEM (Dulbecco's Modified Eagle's Medium)-based medium - that allows acceptable differentiated morphology of the tissue for ~ 5 days upon receipt by end users.

The EPISKIN™ RHE/L/13 human skin equivalent kit was purchased from SkinEthic Laboratories (Lyon, France). The RHE/L/13 tissue constructs are 1.07 cm² supplied with enough MM to allow acceptable tissue differentiation (www.episkin.com). Upon receipt, the EPISKIN™ and EpiDerm™ tissues were equilibrated overnight with their MM at 5% CO₂ and 37 °C before use in the permeation experiments.

Fresh excised human upper breast skin was obtained via Caltag Medsystems Ltd. (Buckingham, UK) from 3 consented female adults (aged 36, 33 and 37 years) following plastic surgery. Selection criteria included: Caucasian, no stretchmarks, no scars and no hair. Full thickness skin without adipose tissue and an overall thickness of 550 ± 80 µm was used. Upon receipt, the *ex vivo* skin samples were equilibrated for 1 hour with 3 mL of DMEM-based (Sigma-Aldrich, UK) culture medium (Table SI-1) at 5% CO₂ and 37 °C before use in permeation experiments.

Dosing Solutions

According to the OECD guidelines (OECD 2004), two different concentration levels of (I) 5

ng/ μ L and (II) 10 ng/ μ L of each of α -HBCD, β -HBCD, γ -HBCD and TBBP-A (Wellington Laboratories Inc., ON, Canada) were prepared in acetone. Based on the exposed surface area, a net dose of 500 ng/cm² (~7.8 μ M/cm²) and 1000 ng/cm² (~15.6 μ M/cm²) was applied to each of the investigated skin tissues using an appropriate volume (100 μ L) of dosing solutions I and II, respectively. The applied doses fall within the range of potential human exposure to the studied BFRs via contact with indoor dust (Abdallah, et al. 2008a). Moreover, they allow for measurement of expected low percentages (up to 0.01%) of the applied dose in various compartments of the exposure model.

To study the possible effect of the dosing vehicle on the percutaneous penetration of the tested chemicals, target BFRs were dissolved in 3 different dosing vehicles of: (A) acetone, (B) 30% acetone in water, and (C) 20% Tween 80 (Sigma-Aldrich, UK) in water at a concentration of 5 ng/ μ L. Preparation of the higher dosing level (i.e. 10 ng/ μ L) was not possible due to limited solubility of target BFRs in vehicles (B) and (C).

Permeation assay protocol

The permeation experiments were performed using the static set-up approach (Figure 1). Skin tissues were mounted in standard Franz-type permeation devices with *stratum corneum* facing up. Based on the recommendation of the 3D-HSE providers, the EpiDerm™ tissues were mounted in specifically designed MatTek™ permeation devices (MatTek Corporation, Ashland, MA), the EPISKIN™ tissues were mounted in special inserts constructed for this model (SkinEthic Laboratories, Lyon, France), while excised human skin tissues were mounted in standard glass Franz cells.

All experiments were performed in triplicate. Following 30 minutes equilibration, the tested chemicals were applied onto the skin surface in the donor compartment. A DMEM-based culture medium (Table SI-1) was used as receptor fluid, maintained at 32 \pm 1 °C and

172 magnetically stirred. To comply with the OECD guidelines, 5% bovine serum albumin (BSA)
173 was added to the receptor fluid (Table SI-1) to enhance the solubility of target analytes, while
174 the levels of test compounds in the donor solutions were chosen to ensure that the
175 concentrations in the receptor fluid during the experiment did not exceed 10% of the
176 saturation solubility.

177 At fixed time points (0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 12, 18, 20 and 24 h), aliquots of the
178 receptor fluid (2 mL) were collected from the receptor compartment and immediately
179 replaced with fresh fluid. After 24 hours, the entire receptor fluid was collected and the skin
180 surface washed thoroughly with cotton buds impregnated in (1:1) hexane:ethyl acetate (5
181 times). The tissues were removed from the permeation devices and both the donor and
182 receptor compartments were washed separately (5 x 2 mL) with (1:1) hexane:ethyl acetate.
183 All samples were stored at -20 °C until chemical analysis.

185 *Sample extraction and chemical analysis*

186 Each permeation assay generated five different types of samples comprising: receptor fluid at
187 various time points, skin tissue, cotton buds (used to thoroughly wipe the skin surface), donor
188 and receptor compartment washes.

189 The receptor fluid, skin tissue and cotton bud samples were extracted according to a
190 previously reported QuEChERS-based method (Abdallah, et al. 2015b) (more details in the
191 supplementary data section).

192 The donor and receptor compartment washes were spiked with 30 ng of the ¹³C-labeled
193 internal standard mixture prior to direct evaporation under a gentle stream of N₂. Target
194 analytes were reconstituted in 100 µL of methanol containing 100 pg/µL d₁₈- α-HBCD used
195 as recovery determination (syringe) standard for QA/QC purposes.

196 Instrumental analysis was carried out using an LC-MS/MS system composed of a dual pump

Shimadzu LC-20AB Prominence liquid chromatograph equipped with SIL-20A autosampler, a DGU-20A3 vacuum degasser coupled to a Sciex API 2000 triple quadrupole mass spectrometer. Details of the multi-residue analytical methodology used for separation and quantification of the studied BFRs can be found elsewhere (Abdallah and Harrad 2011), with a brief description provided as supplementary data.

Data analysis and statistical methods

A quantitative description of test compound permeation through the skin barrier is obtained from Fick's first law of diffusion as follows (Niedorf, et al. 2008):

$$J_{ss} = \frac{\Delta m}{\Delta t \cdot A} = \frac{D \cdot K \cdot \Delta C}{\Delta x} \quad (1)$$

Where J_{ss} = steady-state flux [$\text{ng}/\text{cm}^2 \cdot \text{h}$]; Δm = permeated mass [ng]; Δt = time interval [h]; D = diffusion coefficient [cm^2/h]; K = partition coefficient; A = area [cm^2]; Δc = concentration difference [ng/cm^3]; Δx : thickness of membrane [cm].

When using infinite-dose configurations, i.e. in which the donor concentration far exceeds the concentration in the receptor compartment ($C_D \gg C_A$), ΔC can be replaced by the known donor concentration, C_D , and the permeated mass per time assumed constant. Therefore, the apparent permeation coefficient (P_{app}), which represents an independent measure of the membrane resistance against permeation of the examined substance, can be calculated as:

$$P_{app} = \frac{J_{ss}}{C_D} \quad (2)$$

For each permeation experiment, cumulative amounts of the permeated compounds in the receptor fluid per unit area (ng/cm^2) were plotted versus time (hours). Steady state conditions were indicated by a linear regression line ($R^2 \geq 0.9$), the slope of which represents the flux (J_{ss}). Determination of the start and upper boundary of the linear range (i.e. steady state conditions) was achieved according to the method described by Niedorf et al. (Niedorf, et al.

2008) (a summary flow chart is provided in figure SI-2).

Results are presented as the arithmetic mean of 3 replicates \pm standard deviation (SD). Statistical analysis was performed using SPSS 13.0 software package. Differences in skin permeation were evaluated by the paired student t-test between 2 datasets. A Games-Howell test was used for analysis of variance (ANOVA) among several datasets with equal variances not assumed; $p < 0.05$ was regarded to indicate a statistically significant difference.

QA/QC

Several stages of QA/QC measurements were performed to check the performance of permeation assay protocol. A “field” blank, comprising a skin tissue exposed to solvents only and treated as a sample, was performed with each sample batch ($n=9$). None of the studied compounds were above the limit of detection (LOD) in the field blank samples. Good recoveries of the ^{13}C -labeled internal standards ($> 80\%$) were obtained indicating high efficiency of the extraction method (Table SI-3).

Based on the guidelines of EPISKINTM and EpiDermTM models, the viability of the tissue was tested by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using a standard kit purchased from each provider. Acceptable MTT results (i.e. Formazan concentration ≥ 1.5 mg/ml) were achieved following 24 hours of exposure. Both positive and negative control experiments were carried out alongside each sample batch. Positive controls involved the exposure of the test tissue to Triton-X-100 which showed $\sim 100\%$ permeation ($n=5$; $97 \pm 4\%$), while negative controls showed 0% penetration of decabromodiphenyl ethane after 24 hours exposure. The integrity of the skin membrane was tested using the standard trans-epidermal electrical resistance (TEER) and methylene blue (BLUE) standard methods (Guth, et al. 2015). One excised human skin patch failed the membrane integrity test; hence its results were excluded from this study.

Results and Discussion

Mass balance and absorbed fractions

The efficiency of the experimental approach was investigated using a mass balance exercise. Results revealed good overall recoveries (>85%) for the target compounds using different permeation devices (Table 1). However, the use of specifically-designed permeation devices for the EPISKIN™ and EpiDerm™ models minimized the formation of air bubbles underneath the skin surface and reduced the handling-time and operator involvement during sampling of the receptor fluid at different time points.

For simplicity, results of the permeation experiments were grouped under three major compartments: The directly absorbed dose (cumulative concentration in the receptor fluid over 24 h + receptor compartment rinse), the skin (concentration in the skin tissue after 24 h) and the unabsorbed dose (concentration in the skin surface wipes after 24 h + donor compartment rinse). Experimental results revealed higher permeation of all target compounds in the following order: EpiDerm™ > EPISKIN™ > Human *ex vivo* skin at the two concentration levels studied (Table 1 and Table SI-4). However, statistical analysis showed no significant differences ($P > 0.05$) among the levels of target analytes in the 3 major compartments of the examined tissues. Border line statistical significances ($P = 0.053$ and 0.056) were observed between the results of human *ex vivo* skin and those of EpiDerm™ for β -HBCD and EPISKIN™ for TBBPA, respectively. The EpiDerm™ model displayed the largest permeation difference from human *ex vivo* skin with ~25% increase in the permeated dose of β -HBCD over 24 hours exposure.

Previous studies comparing percutaneous permeation of chemicals through different *in vitro* models reported substantial inter-model differences. A 7-fold higher flux was observed for 11 pesticides across *in vitro* rat skin compared to human skin (van Ravenzwaay and Leibold

2004). For triclosan, a 3-fold higher dermal absorption in rat compared to human skin was observed, while an 8-fold increase in the absorbed dose was reported for BDE-47 (Roper, et al. 2006). Mouse skin showed higher permeability to several chemicals, *in vitro*, than either rat, pig or human skin (Hughes, et al. 2001). A comparative study conducted in 2006 according to OECD guidelines reported less penetration of testosterone in pig and bovine skin (0.07 and 0.13 % of applied dose) compared to human skin (0.32 %), while EPISKIN™ and EpiDerm™ models showed higher permeations (0.53 and 2.36, respectively) (Schafer-Korting, et al. 2006). It is noteworthy that both 3D-HSE producers claim that their skin models were further developed since 2006 to improve the barrier function. Hence the EPISKIN™ and EpiDerm™ models used in this study are listed under the “enhanced barrier function” category, which is different from those used in the 2006 study. Another well-designed study reported higher diffusion of radiolabeled bisphenol A (BPA) through pig ear skin (65%) compared to human skin (45%), although the difference was not statistically significant at the 95% confidence level (Zalko, et al. 2011).

Investigation of the directly absorbed dose through the tested skin models showed a uniform pattern of increasing permeation in the following order: TBBP-A > α -HBCD > β -HBCD > γ -HBCD (Figure 2). This is generally in line with the physicochemical properties of the tested compounds, where TBBP-A has a lower mass and higher water solubility than HBCDs (Table SI-6). Furthermore, a statistically significant correlation ($P < 0.05$) was observed between the 24 h cumulative absorbed dose and the log K_{OW} (Table SI-6) of the studied BFRs in all the tested *in vitro* models. This highlights the influence of physicochemical properties on the human dermal bioavailability of a chemical.

Dermal flux (J_{ss}) and permeation coefficients (P_{app})

A plot of the cumulative absorbed mass of each target compound (ng/cm²) against time (hours) was used to estimate the J_{ss} (ng/cm².h) for each target BFR and the P_{app} (cm/h) for the examined skin models (Table 2). The steady state range of the curve was identified according to the method reported by Niedorf et al. (Niedorf, et al. 2008), with a minimum of 5 data points in the linear range required to establish each curve (Figure SI-3, Table SI-5). Following the application of a test compound to the skin, it needs to partition into and diffuse through the skin before reaching the receptor fluid. This results in a lag-time, t_{lag} , with non-detectable flux. The t_{lag} is represented by the time intercept (i.e. x-axis intercept) of the regression line over the steady-state region of the permeation curve (Figure SI-3). Hence, t_{lag} can be calculated from equation 3:

$$t_{lag} = \frac{b_0}{J_{ss}} \dots \dots \dots (3)$$

Where b_0 refers to the y-axis intercept of the linear regression line and J_{ss} is the slope. Steady state flux (J_{ss}) provides quantitative description of a xenobiotic permeation through the dermal barrier. This is expressed as the rate (ng/ cm². h) by which the tested chemical traverses the skin tissue to reach the receptor fluid (Niedorf, et al. 2008). With γ -HBCD showing lowest percutaneous penetration and TBBPA the highest, J_{ss} of the studied BFRs ranged from 0.8 - 1.5 ng/ cm². h, 0.9 - 1.5 ng/ cm². h and 0.7 - 1.3 ng/ cm². h for the EPISKINTM, EpiDermTM and human *ex vivo* skin, respectively (Table 2). Interestingly, α -HBCD showed a consistently higher flux across skin than γ -HBCD at the studied doses (Table 2). This indicates a higher dermal bioavailability of α -HBCD compared to the β - and γ - isomers. In addition to slower biotransformation rates (Abdallah, et al. 2014) and higher uptake from the gastrointestinal tract (Abdallah, et al. 2012), the greater dermal bioavailability of α -HBCD is likely a contributory factor in the dramatic shift of the HBCD isomeric profile from predominantly γ -HBCD in the commercial formulations and abiotic samples to a predominance of α -HBCD in biota (Covaci, et al. 2006).

The estimated P_{app} values indicate more resistance of human *ex vivo* skin to the penetration of target BFRs than the EPISKIN™ and EpiDerm™ models. However, this difference was not statistically significant. In addition, both 3D-HSE models and human *ex vivo* skin displayed increasing resistance to the penetration of BFRs in the same order of γ -HBCD > β -HBCD > α -HBCD > TBBP-A.

The lipophilic nature, low polarity and low water solubility of the studied BFRs are manifested by long lag times (> 30 minutes; Table 2), which suggests that frequent hand washing may reduce human exposure to HBCDs and TBBPA via dermal contact. This is generally in line with the results of Watkins et al. who found that adults washing their hands fewer than four times/day had, on average, 3.3 times more pentaBDE in their handwipes compared with those who washed their hands four or more times/day and concluded that frequent hand washing may decrease exposure to PBDEs via dermal contact (Watkins, et al. 2011).

Effect of dosing vehicle

Several studies in the pharmaceutical and cosmetic sectors have highlighted the influence of dosing vehicle on the percutaneous penetration of chemicals. However, these experiments were exclusively based on aqueous solutions and topical emulsions (Schaefer-Korting, et al. 2008b). Very little is known about the quantitative effects of organic-based vehicles on the dermal penetration of xenobiotics. In general, a vehicle may hydrate the stratum corneum (SC), extract critical barrier components out of the skin, or damage the skin because it is a strong acid or base. Removing SC lipids may increase percutaneous absorption of drugs. Many organic solvents (e.g. chloroform and methanol) are employed to delipidize the skin, which increases the permeability of hydrophilic - but not lipophilic – compounds (Chiang, et al. 2012).

Since BFRs are highly lipophilic compounds with very low water solubility (Table SI-6), the few studies on their dermal absorption used organic vehicles to dissolve the target analytes. Hughes et al. used tetrahydrofuran (THF) as a vehicle for BDE-209 (Hughes, et al. 2001), while Roper et al. used acetone for dissolving BDE-47 (Roper, et al. 2006). In the current study, acetone was selected as the major dosing vehicle. This was based on its ability to dissolve the test compounds at the desired levels and its minimal effect on skin barrier functions. Abrams et al. studied the effect of various organic solvents on the trans-epidermal water loss (TEWL) as an indicator of skin barrier. Both acetone and hexane showed no significantly different effects than water, while a mixture of chloroform : methanol (2:1) caused the greatest significant increase in TEWL (Abrams, et al. 1993).

To further investigate the potential effect of the dosing vehicle on percutaneous penetration of BFRs, human *ex vivo* skin and the EPISKIN™ model were exposed to 500 ng/cm² of target BFRs in each of : (A) acetone, (B) 30% acetone in water, and (C) 20% Tween 80 in water for 24 h. Results revealed higher levels of target compounds were absorbed from vehicle C, which was more evident for TBBP-A and α -HBCD compared to β - and γ -HBCDs (Figures 3 and SI-4). This is in agreement with the reported enhancement of the dermal absorption of testosterone in the presence of surfactants including miglyol and Tween 80 (Schaefer-Korting, et al. 2008b).

Although the differences in permeation of the studied BFRs from the tested vehicles lacked statistical significance, the enhanced permeation of TBBP-A and α -HBCD (Figure 3) in the presence of Tween 80 is potentially pertinent within the context of human exposure. This is owing to the presence of natural surface active agents in human skin surface film (sweat/sebum mixture) (Stefaniak, et al. 2010), which may influence the dermal absorption of these BFRs. Therefore, detailed study of the effect of human skin surface film on the dermal uptake of various BFRs appears warranted in the near future. In conclusion, the data

presented here demonstrate the validity of the 3D-HSE models for studying human dermal uptake of BFRs and related environmental contaminants.

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Supplementary data

Further details of the analytical methodology, quality assurance/quality control parameters and distribution of target BFRs in different compartments of the *in vitro* diffusion system are available as supplementary data.

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Tables

Table 1: Distribution of target BFRs (expressed as % of exposure dose) in different fractions of the *in vitro* diffusion system following 24 hour exposure to 500 ng/cm² of α -, β -, γ -HBCDs and TBBP-A in acetone.

	α -HBCD	β -HBCD	γ -HBCD	TBBP-A
EPISKIN™				
Receptor fluid (24h)	5.81 ± 1.04	3.86 ± 0.78	3.42 ± 0.94	6.29 ± 0.65
Receptor rinse	0.10 ± 0.02	0.07 ± 0.02	0.11 ± 0.02	0.41 ± 0.28
Directly absorbed fraction	5.90 ± 1.06	3.94 ± 0.82	3.46 ± 0.96	6.70 ± 0.92
Skin-Epidermis (Depot)	30.06 ± 2.42	27.18 ± 2.28	23.66 ± 3.16	24.18 ± 2.54
Skin wash (unabsorbed)	44.34 ± 4.04	51.47 ± 3.72	56.82 ± 4.58	53.53 ± 3.46
Donor rinse (unabsorbed)	5.13 ± 0.64	3.16 ± 0.82	2.38 ± 1.06	4.93 ± 2.08
Unabsorbed dose	49.47 ± 4.68	54.63 ± 4.54	59.20 ± 5.64	58.46 ± 5.54
Total Recovery	85.43 ± 8.16	85.75 ± 7.64	86.32 ± 9.76	89.34 ± 9.02
EpiDerm™				
Receptor fluid (24h)	6.35 ± 0.92	4.02 ± 1.04	3.74 ± 0.82	6.44 ± 0.59
Receptor rinse	0.11 ± 0.04	0.10 ± 0.08	0.09 ± 0.04	0.34 ± 0.16
Directly absorbed fraction	6.46 ± 0.94	4.13 ± 1.12	3.82 ± 0.86	6.78 ± 0.74
Skin-Epidermis (Depot)	28.19 ± 3.18	24.39 ± 2.22	21.02 ± 3.52	23.79 ± 2.42
Skin wash (unabsorbed)	45.73 ± 4.02	53.91 ± 3.44	58.84 ± 4.38	55.04 ± 4.29
Donor rinse (unabsorbed)	5.07 ± 0.62	2.39 ± 0.52	1.97 ± 0.74	4.11 ± 1.27
Unabsorbed dose	50.80 ± 4.64	56.30 ± 3.96	60.81 ± 5.12	59.15 ± 5.56
Total Recovery	85.45 ± 8.76	84.82 ± 7.30	85.65 ± 9.50	89.72 ± 8.72
Human <i>ex vivo</i> skin				
Receptor fluid (24h)	4.88 ± 1.44	3.21 ± 1.06	3.01 ± 1.02	5.37 ± 0.65
Receptor rinse	0.07 ± 0.02	0.11 ± 0.02	0.06 ± 0.02	0.21 ± 0.28
Directly absorbed fraction	4.95 ± 1.44	3.32 ± 1.06	3.07 ± 1.48	5.57 ± 0.92
Skin-Epidermis (Depot)	30.59 ± 2.28	27.82 ± 2.38	24.16 ± 2.24	24.71 ± 2.96
Skin wash (unabsorbed)	47.05 ± 4.44	51.19 ± 4.68	56.48 ± 3.28	56.53 ± 4.46
Donor rinse (unabsorbed)	5.23 ± 1.48	3.37 ± 1.02	2.07 ± 0.66	3.83 ± 2.08
Unabsorbed dose	52.28 ± 5.92	54.56 ± 5.70	58.55 ± 3.94	60.37 ± 6.54
Total Recovery	87.82 ± 7.84	85.70 ± 6.28	85.78 ± 7.38	85.65 ± 10.42

Table 2: Steady state flux, permeation coefficient and lag time values estimated for the target BFRs using different *in vitro* skin models.

	Flux (J_{ss}) (ng/cm ² .h)	Permeation coefficient (P_{app}) (cm/h)	Lag time (h)
EPISKIN™			
α-HBCD	1.25	2.50 x 10 ⁻⁰⁴	0.80
β-HBCD	0.84	1.69 x 10 ⁻⁰⁴	1.01
γ-HBCD	0.78	1.56 x 10 ⁻⁰⁴	1.21
TBBPA	1.47	2.93 x 10 ⁻⁰³	0.72
EpiDerm™			
α-HBCD	1.33	2.74 x 10 ⁻⁰⁴	0.77
β-HBCD	0.88	1.77 x 10 ⁻⁰⁴	0.97
γ-HBCD	0.85	1.72 x 10 ⁻⁰⁴	1.13
TBBPA	1.48	2.97 x 10 ⁻⁰³	0.60
Human <i>ex vivo</i> skin			
α-HBCD	1.08	2.16 x 10 ⁻⁰⁴	0.85
β-HBCD	0.74	1.47 x 10 ⁻⁰⁴	1.17
γ-HBCD	0.69	1.37 x 10 ⁻⁰⁴	1.26
TBBPA	1.29	2.58 x 10 ⁻⁰³	0.79

Figures

Figure 1: General outline of the experimental protocol applied for percutaneous permeation experiments.

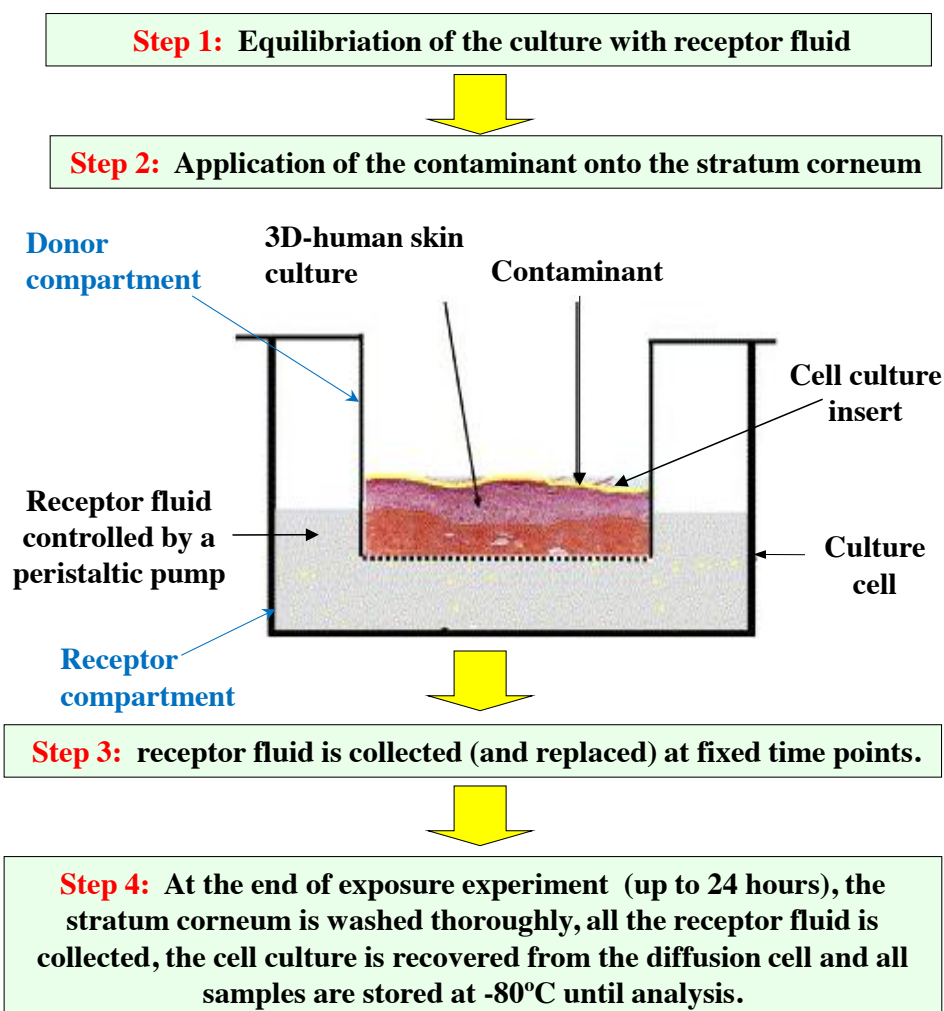


Figure 2: Cumulative dose absorbed into the receptor fluid following exposure of (a) human *ex vivo* skin, (b) EPISKIN™ and (c) EpiDerm™ to 1000 ng/cm² of target BFRs over 24 h.

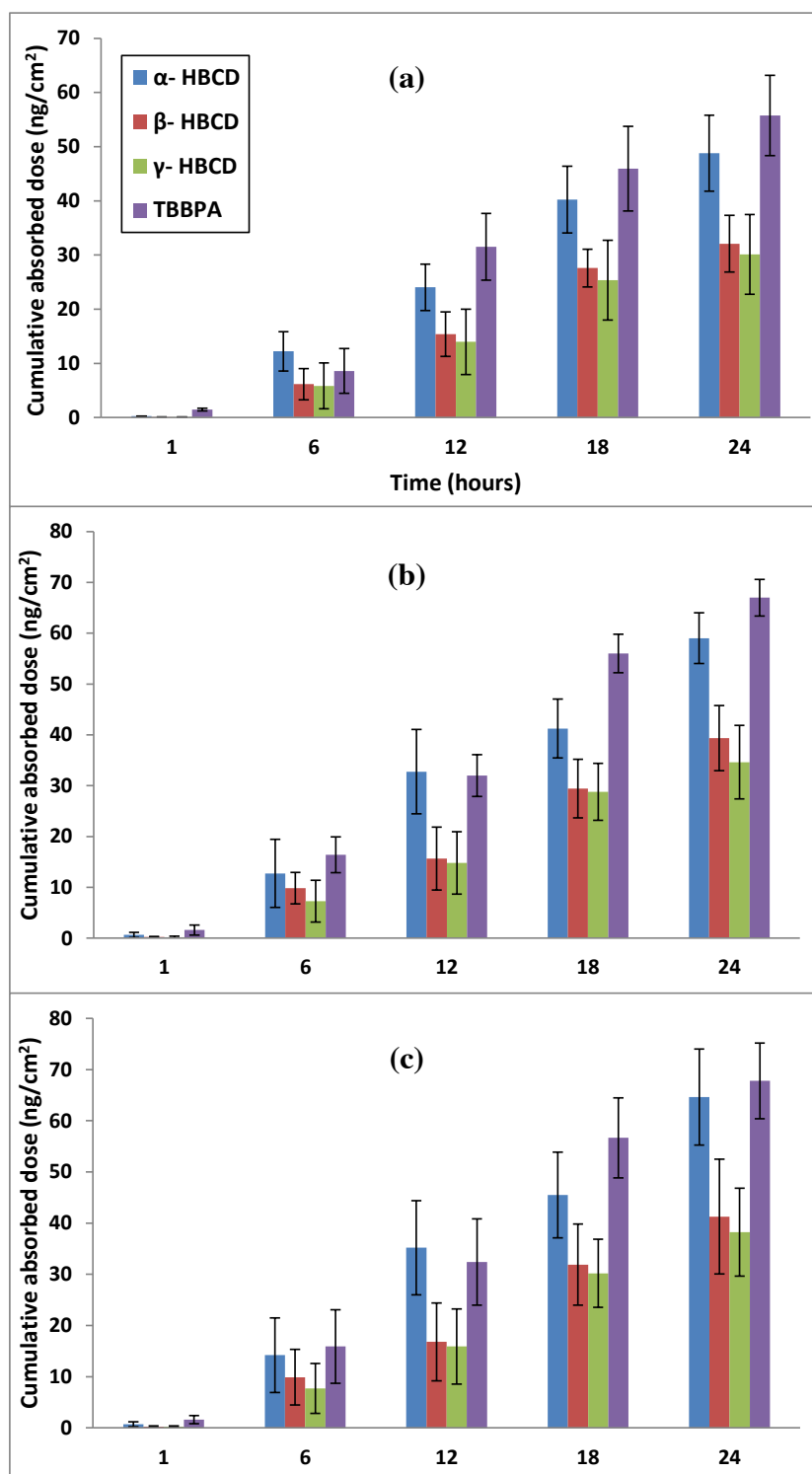
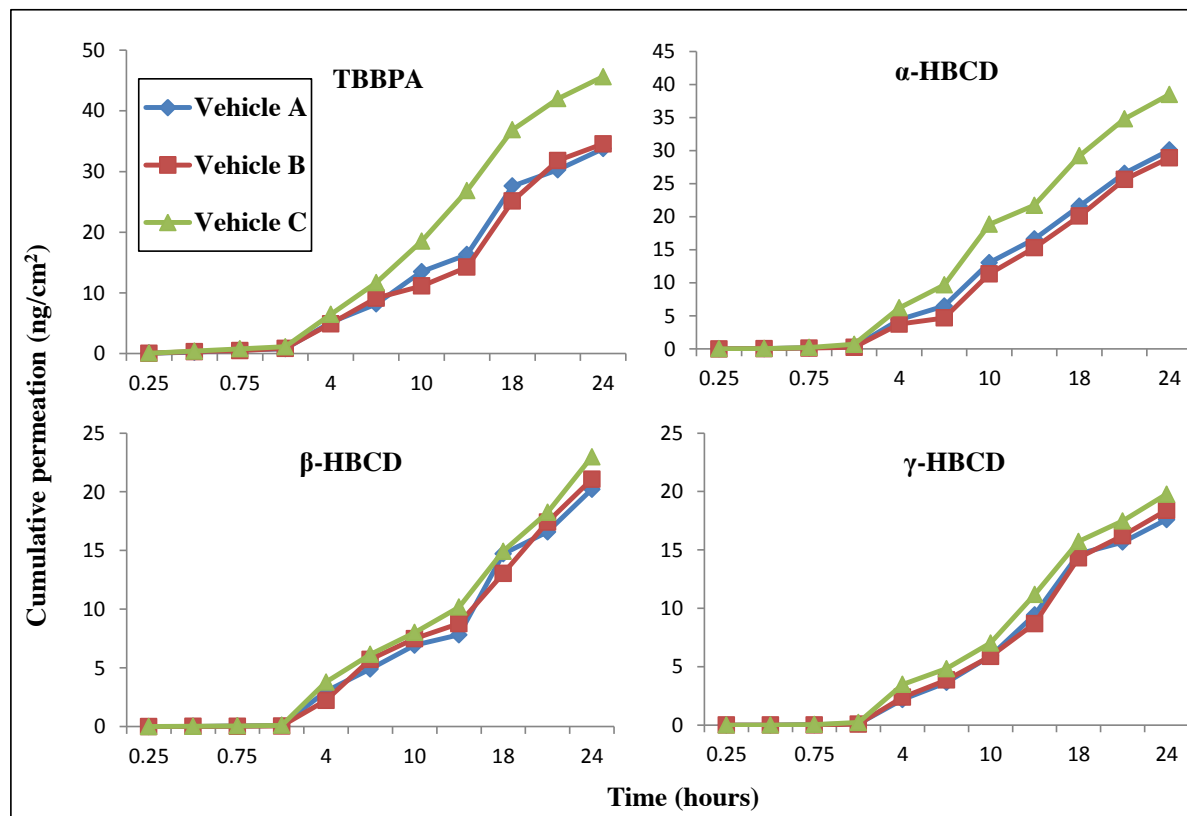


Figure 3: Cumulative permeation (ng/cm²) into the receptor fluid following exposure of EPISKIN™ model to 500 ng/cm² of target BFRs in (A) acetone, (B) 30% acetone in water, and (C) 20% Tween 80 in water for 24 h.



Supplementary Information

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